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The first species of *Aplastodiscus* endemic to the Brazilian Cerrado (Anura, Hylidae)

Bianca V. M. Berneck[1], Ariovaldo A. Giaretta[2], Reuber A. Brandão[3], Carlos A. G. Cruz[4], Célio F. B. Haddad[1]

1 *Departamento de Zoologia, Instituto de Biociências, UNESP, Universidade Estadual Paulista, Campus de Rio Claro, São Paulo, Brazil* **2** *Laboratório de Taxonomia, Sistemática e Ecologia de Anuros Neotropicais. Faculdade de Ciências Integradas do Pontal, Universidade Federal de Uberlândia, UFU, Ituiutaba, Minas Gerais, Brazil* **3** *Laboratório de Fauna e Unidades de Conservação, Departamento de Engenharia Florestal, Universidade de Brasília, Brasília, Brazil* **4** *Departamento de Vertebrados, Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista, Rio de Janeiro, Rio de Janeiro, Brazil*

Corresponding author: *Bianca V. M. Berneck* (bvmberneck@gmail.com)

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Abstract

The genus *Aplastodiscus* includes 14 nominal species in four monophyletic groups with occurrence in the Atlantic Forest and Brazilian Cerrado (Brazilian Savanna) of South America. A recent study reviewed the taxonomy and phylogenetic relationships of the genus and suggested a third species for the *A. perviridis* Group. Herein, on the basis of morphology and advertisement call, we describe this species and test its monophyly. The new species is the only *Aplastodiscus* with endemic occurrence in the Cerrado Biome. In addition, its geographical distribution and conservation status are discussed.

Keywords

Aplastodiscus lutzorum sp. n., Cophomantinae, new species, integrative taxonomy

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Introduction

The genus *Aplastodiscus* includes 14 nominal species in four monophyletic groups (Berneck et al. 2016) with occurrence mainly in Atlantic Forest in Brazil and Argentina (Frost, 2016). The species are stream-breeding treefrogs, usually of green color and medium size. The *A. perviridis* species Group includes *A. perviridis* Lutz 1950 and *A. cochranae* (Mertens 1952), which share, among other characters, bicolored irises, no webbing between toes I and II, and reduced webbing among their remaining toes (Garcia et al. 2001).

Berneck et al. (2016) recently reviewed the taxonomy and phylogenetic relationships of all *Aplastodiscus* species and suggested a third species for the *A. perviridis* Group, the "*Aplastodiscus* sp. 3". This species was previously called *A. perviridis* by the previous authors (Garcia et al. 2001; Bastos et al. 2003; Morais et al. 2012; Valdujo et al. 2012). Herein, on the basis of morphology and advertisement call, this species is described as new, and its monophyly tested. In addition, its geographical distribution and conservation status are discussed.

Materials and methods

Descriptions of adults and their calls

The following measurements follow Duellman (1970):

SVL snout-vent length,
HL head length,
HW head width,
ED eye diameter,
TD tympanum diameter,
END eye-nostril distance,
IOD interocular distance,
THL thigh length,
FL foot length; including tarsus.

However, the tibia length (**TBL**) follows Heyer et al. (1990). Measurements are in millimeters and were taken with a digital caliper and, except for SVL, HL, HD, THL, and TBL, under a stereomicroscope. The webbing formula follows Savage and Heyer (1967) and Myers and Duellman (1982). Adult males were collected while calling and/or recognized by the presence of vocal slits.

The adult specimens are housed in the following Brazilian collections: Célio F. B. Haddad collection (**CFBH**) at the Universidade Estadual Paulista, Rio Claro, SP; Coleção Herpetológica da Universidade de Brasília (**CHUNB**) at the Universidade de Brasília, Brasília, DF; Museu de Ciências Naturais da Pontifícia Universidade Católica

de Minas Gerais (**MCN-AM**), Belo Horizonte, MG; and Coleção de Anuros da Universidade Federal de Uberlândia (**AAG-UFU**), Uberlândia, MG.

Males of the new species were recorded in the Municipality of Alto Paraíso de Goiás, Goiás State (N = 6) and Brasília, Distrito Federal. For comparative purposes, males of *A. perviridis* were recorded at the type-locality (N = 5), in Serra da Bocaina, São José do Barreiro, São Paulo State. Calls were recorded with a Marantz PMD 671, a Boss BR-864 (both with a Sennheiser ME67/K6 microphone) or a MicroTrack (ME66/K6 microphone), all set at 44.1 kHz and 16-bit resolution. Calls were recorded from 50 to 150 cm from calling males, and 10 to 15 calls were analyzed for each male. Acoustic variables were analyzed with RAVEN PRO 1.5, 64-bit version (Bioacoustics Research Program 2014); terminology used for call features were according to Raven's manual (Charif et al. 2010). A 500 Hz high pass filter was applied prior to call analyses and figuring to reduce wind noise interference. Sound figures were obtained with the SEEWAVE 1.6.4 (Sueur et al. 2008) R package (R Development Core Team, 2012, v. 2.15.1), settings used were the Hanning window, 85% overlap, and 256 points resolution. Measured call parameters were: 1) call duration (CD), 2) peak of dominant frequency (PDF), 3) lower dominant frequency (LDF), 4) higher dominant frequency (HDF), 5) time to frequency peak (TFP) (expressed as % of call duration). All calls used in descriptions are housed at the AAG-UFU collection (Suppl. material 1, Table 1).

Laboratory protocols and genetic distance calculation

The extraction of DNA was carried out using ethanol-preserved tissues and the DNeasy isolation kit (Qiagen, Valencia, CA, USA). We carried out DNA amplification in a 25 μL volume reaction using master mix Fermentas Taq Polymerase and reagents. The Polymerase chain reactions (PCR) included an initial denaturing step of 30s at 94 °C, followed by 35 cycles of amplification with a final extension step at 72 °C for 6 min. The products of PCR were sent for sequencing to Macrogen, South Korea. We sequenced DNA fragments in both directions to minimize potential errors. The chromatograms were read and edited using SEQUENCHER 3.0 (Gene Codes, Ann Arbor, MI, USA) and complete sequences were edited with MEGA 6.06 (Tamura et al. 2013). The distance estimations of genetic p-distances were calculated in MEGA 6.06 for the regions delimited by the primers 16sAR (Palumbi et al. 1991), Wilk2 (Wilkinson et al. 1996), and COI (Jungfer et al. 2013), considering d:transitions + transversions, uniform rates among sites, and gaps/missing data as complete deletion. A list of vouchers, GenBank accession numbers, and locality data is available in Suppl. material 2.

Phylogenetic analysis and taxon sampling

Berneck et al. (2016) studied *Aplastodiscus* in a wider context and consequently included only one specimen of the species described here. Therefore, we carried out a reduced

phylogenetic analysis that included all terminals of the *A. perviridis* Group analyzed by Berneck et al. (2016) and four specimens of the species described here. As outgroups, we included two terminals of the *A. albosignatus* Group and two of the *A. albofrenatus* Group, rooting the tree in the *A. sibilatus* Group (see Berneck et al. 2016). The dataset used for the analysis were the fragments delimited by the primers 16sAR, Wilk2, and COI forward and reverse.

Sequence alignments were performed in Clustawl (Thompson et al. 1994) under MEGA 6.06. For the phylogenetic analysis T.N.T Willi Hennig Society Edition was employed (Goloboff et al. 2008) with searches by "new technology", search level 50, sectorial searches included, tree drift, and tree fusing (Goloboff 1999), hitting the best length 100 times. Parsimony Jackknife absolute frequencies (Farris et al. 1996) were also estimated using "new technology" and requesting 10 hits, driven searches, and 1000 replicates. Edition of trees were made with FIGTREE (Rambaut 2014).

Results

***Aplastodiscus lutzorum* sp. n.**
http://zoobank.org/C506C42B-20FF-41B6-9E5F-177E50C3415F
Figs 1, 2

Aplastodiscus perviridis Garcia et al. (2001)
Aplastodiscus perviridis Bastos et al. (2003)
Aplastodiscus perviridis Morais et al. (2012)
Aplastodiscus perviridis Valdujo et al. (2012)
Aplastodiscus sp. Santoro and Brandão (2014)
Aplastodiscus sp. 3 Berneck et al. (2016)

Holotype. (Figs 1 and 2) AAG-UFU 864. Adult male collected at Fazenda São Bento (14°09'39"S, 47°34'55"W; 1150 meters above sea level), Municipality of Alto Paraíso de Goiás, Goiás State, Brazil, on 12 December 2011, by A. A. Giaretta and K. G. Facure.

Paratypes. CFBH 22777–80, four adult males collected at Fazenda Água Limpa, Brasília, Distrito Federal (15°56'55.45"S; 47°56'17.83"W) on 18 February 2009. AAG-UFU 863, 865-867 and AAG-UFU 1639 collected on December 2012, AAG-UFU 3343 on 29 November 2013, AAG-UFU 3350–51, 5073–76, 5091, AAG-UFU 0867, adult female, all collected with the holotype. CHUNB 17015–17016 adult males collected on 31 December 1995 at Alexânia, Goiás (16°5'42.00"S; 48°31'20.60"W), CHUNB 17018, adult male collected on 12 December 1985 at Alexânia, Goiás, and CHUNB 74504–74508 adult males from Fazenda Água Limpa, Brasília, Distrito Federal, collected on March 2013. All localities are in Brazil.

Referred specimens. All males. MCN-AM 8809–12 and 8767–72 from AHE Queimado, Unaí, Minas Gerais (16°20'55.51"S; 46°52'48.93"W), collected on February–March 2007.

Figure 1. Holotype of *Aplastodiscus lutzorum* sp. n. (AAG-UFU 864). **A** Lateral view of head **B** dorsal view of head **C** plantar view **D** palmar view. Scale bar 12 mm.

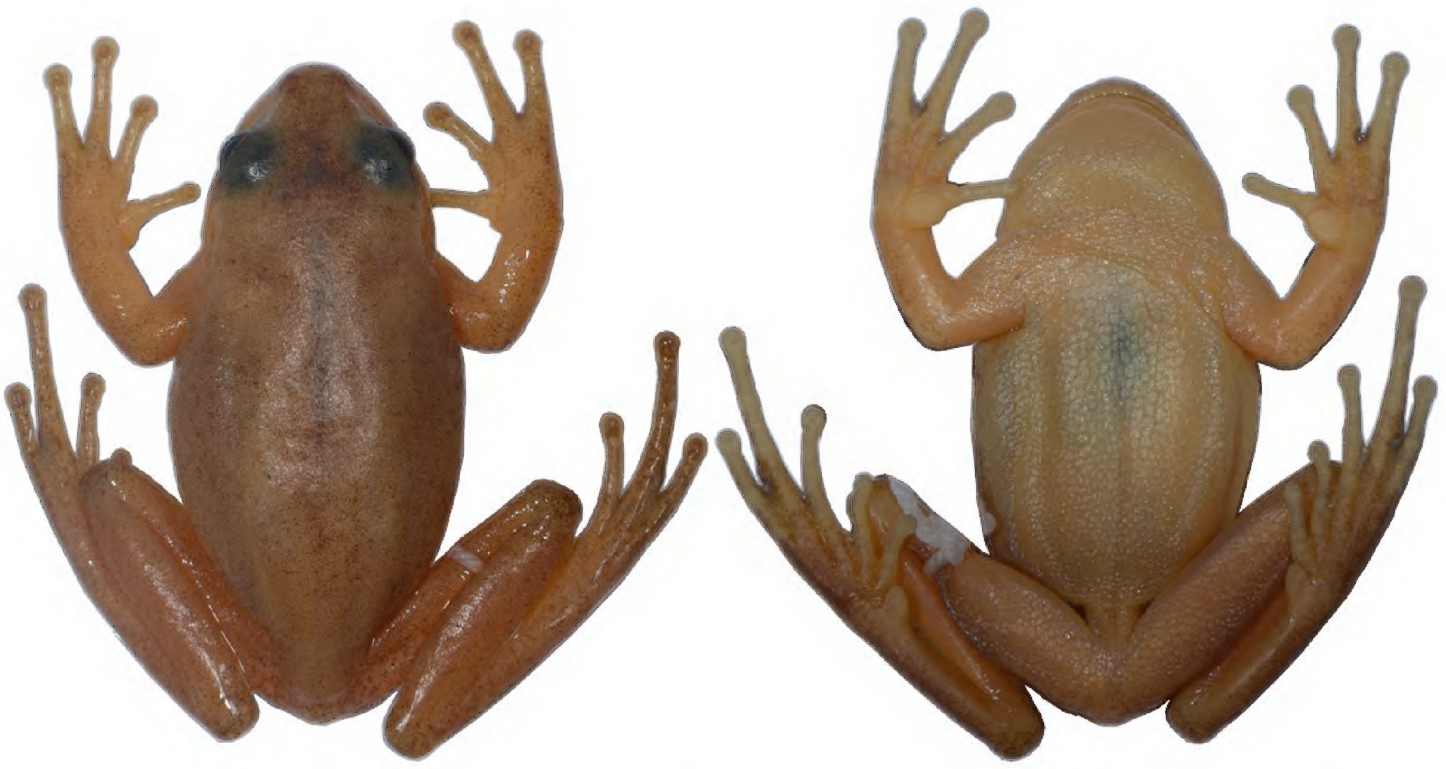

Figure 2. Dorsal and ventral views of the Holotype of *Aplastodiscus lutzorum* sp. n. (AAG-UFU 864).

Diagnosis. *Aplastodiscus lutzorum* sp. n. belongs to the *A. perviridis* Group and thus bears bicolored irises, lacks webbing between toes I and II, has reduced webbing among the remaining toes, and lacks peri-cloacal ornamentation. The new species is diagnosed by its small SVL for the *A. perviridis* Group (30–36 mm) and by the advertisement call 2.5 times longer.

Description of holotype. Adult male: head 20% wider than long; snout rounded in profile, nearly rounded in dorsal view; *canthus rostralis* curved; loreal region concave; nostrils ovoid; internarial region grooved. Supratympanic fold distinct, from posterior corner of orbit to insertion of forearm; tympanum distinct, almost circular; tympanum diameter 48.5% of eye diameter. Upper eyelid smooth as the dorsum. Thoracic fold just discernible. External vocal sac single, subgular, and expanded. Fingers long, slender, no lateral fringe, bearing discret round terminal discs; relative lengths of fingers I, II, IV, III; similar sized discs on fingers II, III and IV, larger than disc of Finger I. Diameter of disc of Finger III equals to diameter of Toe IV, about 42% eye diameter. Subarticular tubercles well defined, rounded; supernumerary tubercles poorly defined on palm, rounded; inner metacarpal tubercle large, rounded, about four times the size of subarticular tubercles; other metacarpal tubercle barely defined; spine of prepollex absent; no nuptial pads; ulnar crest barely defined. Hand webbing formulae: I-II 2^{-}-3^{-} III 2^{+}-2 IV. Tarsal texture smooth; tarsal fold discrete, extending to the entire length of tarsus; heel smooth; inner metatarsal tubercles large, elongate, three times the size of foot subarticular tubercles; outer metatarsal tubercle absent; subarticular tubercles distinct, large, and rounded, diameter about 3/4 width of terminal disc on the same toe; supernumerary tubercles absent; toes long, slender, without lateral fringe; toes bearing rounded discs, smaller in diameter to those of fingers II-IV. Foot webbing formula: I 2^{+} - 3^{-} II 2^{+} - $3^{1/2}$ III 2^{+} - 4^{-} IV 3^{+} - 2V. Supra cloacal fold absent. Skin on dorsum smooth; skin on throat, belly, ventral surface of

Table 1. Acoustic variables of the advertisement call of topotypes *Aplastodiscus perviridis* and *A. lutzorum* sp. n. n = number of recorded males.

Call Features	*A. lutzorum* sp. n. (n = 12) Range	Mean (SD)	*A. perviridis* (n = 5) Range	Mean (SD)
Call Duration (seconds)	0.26–0.40	0.32 (0.05)	0.12–0.15	0.13 (0.01)
Higher Frequency (kHz)	2334–2647	2468 (97)	2419–2750	2519 (135)
Lower Frequency (kHz)	1494–1732	1595 (76)	1587–1806	1690 (82)
Dominant Peak (kHz)	1884–2156	2027 (79)	1981–2153	2078 (66)
Time to Frequency Peak (%)	49–70	61 (7)	23–38	34 (6)
Air temperature range	19–22 °C		16–19 °C	
Record hour	20:00–22:00 h		20:30–21:00 h	

Table 2. Measurements (in millimeters) of the type-series of *Aplastodiscus lutzorum* sp. n. Abbreviations are: SVL (snout-vent length), HL (head length), HW (head width), ED (eye diameter), TD (tympanum diameter), END (eye-nostril distance), IOD (interocular distance), THL (thigh length), TBL (tibia length), and FL (foot length).

Measurement	Holotype	Female paratype	Males paratypes N = 25 (Mean)
SVL	34.6	33.7	30.7–36 (33.5)
HL	10.6	11.4	8.8–11.4 (10.5)
HW	11.4	11.1	10.5–12.4 (11.4)
ED	3.3	3.4	3–3.7 (3.4)
TD	2.1	2.4	1.5–2.4 (2)
END	3.2	3.1	1.6–3.3 (2.7)
IOD	5.3	5.7	4.5–5.9 (5.4)
THL	18.1	16.2	12–18.7 (17)
TBL	15.8	16.4	14.2–18.5 (16)
FL	18.3	18.8	14.9–19.6 (17)

thigh, and arm granular. Dorsal and dorsolateral surfaces almost entirely pale yellow with small dark spots or mottles on dorsal surfaces. Belly pale yellow. Measurements of the holotype (mm): SVL 34.6, HL 10.6, HW 11.4, ED 3.3, TD 2.1, END 3.2, IOD 5.3, THL 18.1, TBL 15.8, and FL 18.4 (Table 2).

Color in life of the type-series: Dorsal head surface dark green, almost olive. Dorsal body surface and flanks yellowish green with small and scattered melanophores. The superior third of eye is golden, whereas the inferior 2/3 is red copper. Eye surrounded by a black ring. Vocal sac bluish green. In preservative, colors fade to pale beige and the dorsum shows several dark brown dots, making it darker than other parts of the body. The belly is uniformly pale yellow.

Variation in the type series. The main variation in type series is the body size (Table 2). Small brown chromatophores are along the dorsal skin; but the amount of these chromatophores is variable, ranging from sparse to dense.

Calls. Advertisement calls of *A. lutzorum* sp. n. (Figure 3 and 4, Table 1) are long regularly-spaced single notes released at a mean rate of 39 calls/minute (SD = 8; n = 12

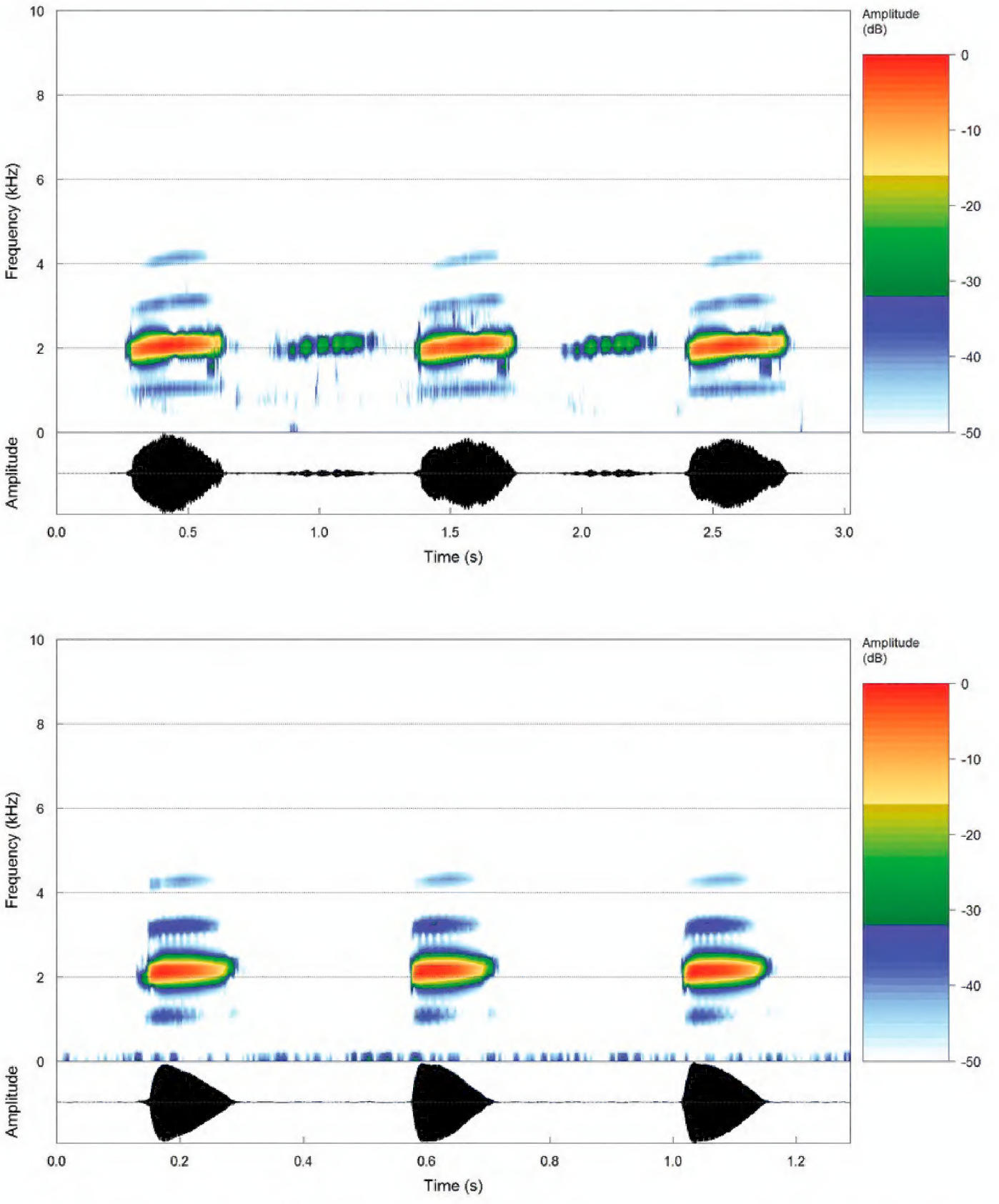

Figure 3. Above, audiospectrogram and oscillogram of three advertisement calls of the holotype of *Aplastodiscus lutzorum* sp. n. (Chapada dos Veadeiros, 12 December 2011, air temperature 20 °C); the background calls are from another male calling in antiphony. Bellow, audiospectrogram and oscillogram of three advertisement call of *A. perviridis* (Serra da Bocaina, 10, January 2012, air temperature 16 °C).

males). Calls resemble a whistle lasting around 0.26–0.40s. Most of the energy is between 1,494–1,732 Hz and 2,334–2,647 Hz, peaking between 1,884–2,156 Hz. Call exhibits an ascending frequency modulation, reaching its maximum between 49–70% of the call duration.

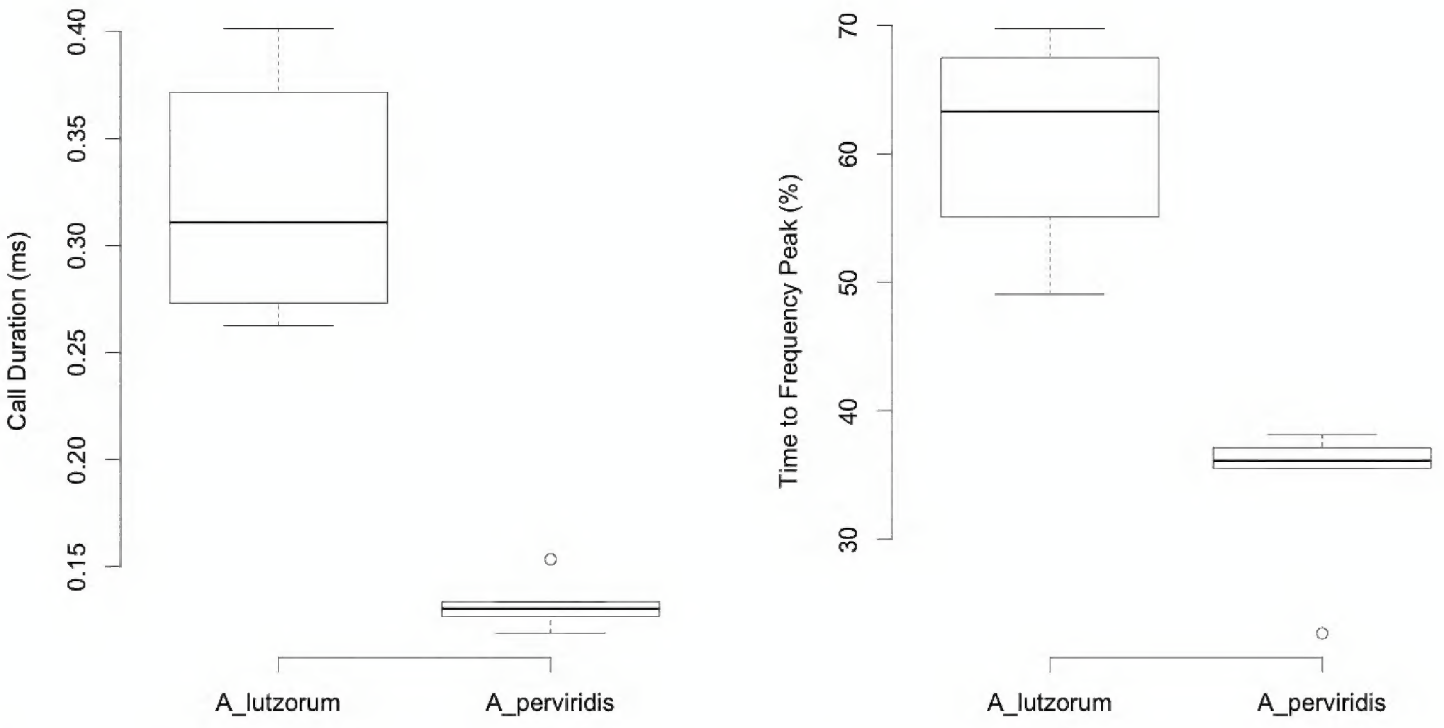

Figure 4. A comparison between duration and frequency peak time of *Aplastodiscus lutzorum* (N = 12 males) and *A. perviridis* (N = 5 males). In both samples, calls are of topotypes.

Aplastodiscus perviridis' advertisement call (Figure 3 and 4, Table 1) is released in groups of 1–11 (mode = 5–7); within groups, call released at a rate of 140/min. Calls resemble a short whistle lasting around 0.12–0.15 s. Most of the energy is between 1,587–1,806 Hz and 2,419–2,750 Hz, peaking between 1,981–2,153 Hz. Call with an ascending frequency modulation, reaching its maximum between 23–38% of the call duration. The advertisement call of *A. cochranae* is described by Garcia et al. (2001) as being barely indistinguishable from the call of *A. perviridis*.

Natural history and geographic distribution. All specimens of *Aplastodiscus lutzorum* sp. n. were collected along gallery forests with scattered buriti (*Mauritia flexuosa*) palm trees within the Cerrado Biome (see also Brandão and Araujo, 2002; Morais et al. 2012; Santoro and Brandão, 2014) (Figure 5). A female bearing large oocytes seen by the transparency of the body walls was found in mid-December and calling males were found from December to March. Males call during the night in proximity of riverine forests (< 2m), perched on leaves or branches from the water level to 5 m high (Figure 6). *Aplastodiscus perviridis* males call during the night along swamps in open areas, perched on grass leaves or bushes bordering streamlets, from 0.5 m to 3 m high. Tadpoles are unknown. Sometimes, the species also uses places with wet and soft mud soil, covered by a layer of dense bush, in places where the forest was removed, but is under secondary growth. Sympatric frog species were *Hypsiboas ericae* (Caramaschi and Cruz, 2000) and *H. albopunctatus* (Spix, 1824). All localities where *A. lutzorum* sp. n. was found are 1000 m above sea level or more.

Etymology. The new species is named after the Brazilian scientists Adolfo and Bertha Lutz, who were pioneers in discovering and studying *Aplastodiscus* and some species of *Hyla* now included in the genus *Aplastodiscus*.

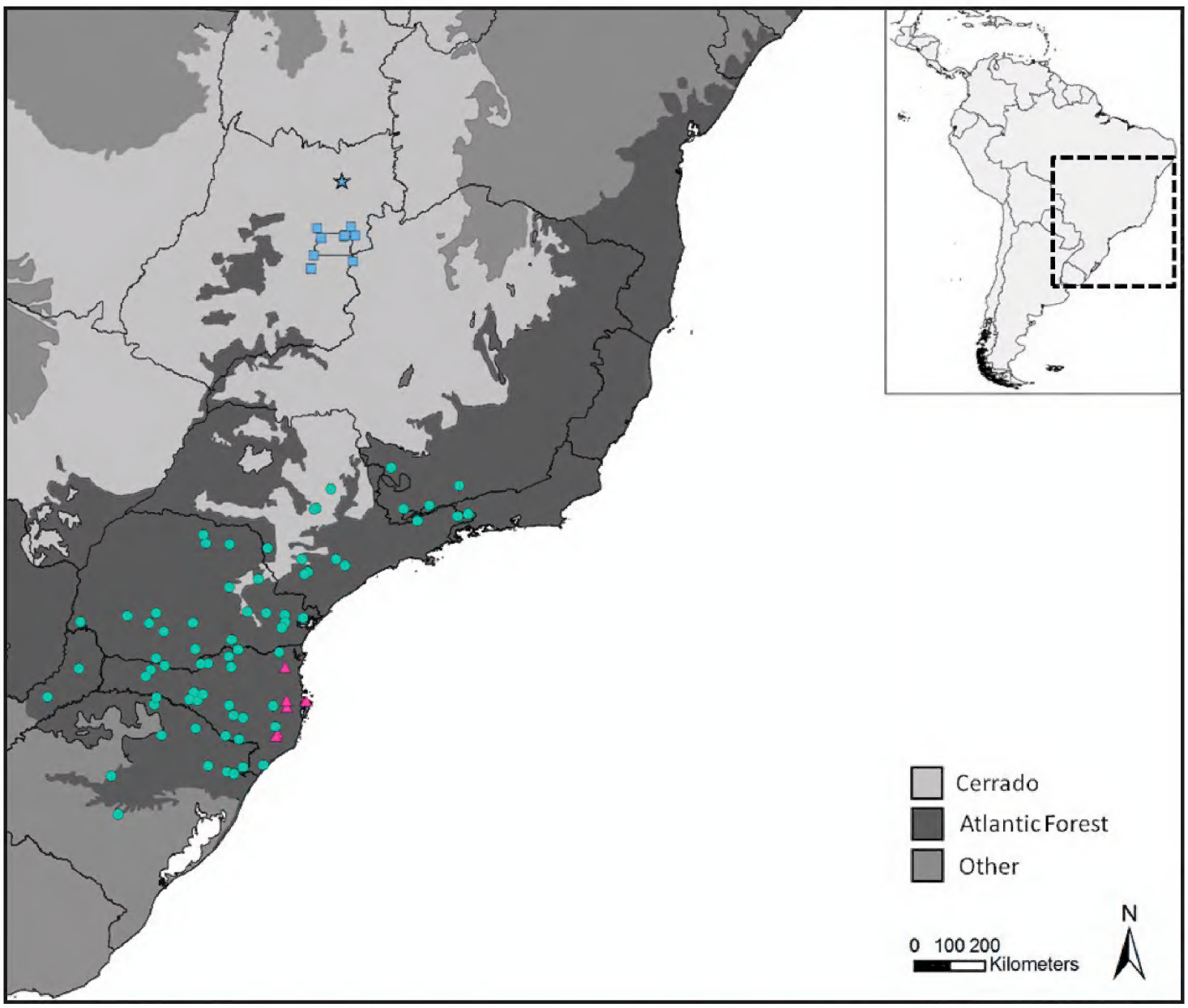

Figure 5. Geographic distribution of *Aplastodiscus cochranae* (pink triangles), *A. perviridis* (green circle), and *A. lutzorum* sp. n. (blue squares, blue star indicates its type-locality). Note that *A. lutzorum* shows a disjunctive distribution regarding the other *Aplastodiscus* species, occurring deep within Cerrado Biome.

Comparison with congeneric species. *Aplastodiscus lutzorum* sp. n. can be distinguished from the species of other groups of *Aplastodiscus* (*A. albosignatus*, *A. albofrenatus,* and *A. sibilatus* groups) by the presence of bicolored irises, the lack of the webbing between toes I and II, the webbing among the remaining toes reduced, and the absence of peri-cloacal ornamentation. The new species is diagnosed from *A. perviridis* and *A. cochranae* by having smaller SVL (30–36 mm SVL in the new species, 38–44.7 mm in *A. perviridis*, and 41–46.4 mm in *A. cochranae*) and longer advertisement calls (0.38–0.42 in new species, 0.14–0.17 in *A. perviridis* and 0.10–0.18 in *A. cochranae*). From *A. cochranae* it can be also distinguished by the green dorsal color in life (*A. cochranae* is brown) and by the absence of a brown line on the loreal region and a white line in the cloacal region (both present in *A. cochranae*). (Figures 1–4; Tables 1 and 2).

Phylogenetic relationships and genetic p-distances. The two DNA fragments aligned and concatenated resulted in a matrix of 1,227pb. Our analysis recovered four most parsimonious trees with 808 steps each (Figure 7). The differences in topology among these trees are the position of *A. lutzorum* sp. n. specimens from different locali-

Figure 6. A The habitat of *A. lutzorum* sp. n.: flooded gallery forests **B** A male in calling activity at Fazenda Água Limpa, Brasília, Distrito Federal, Brazil.

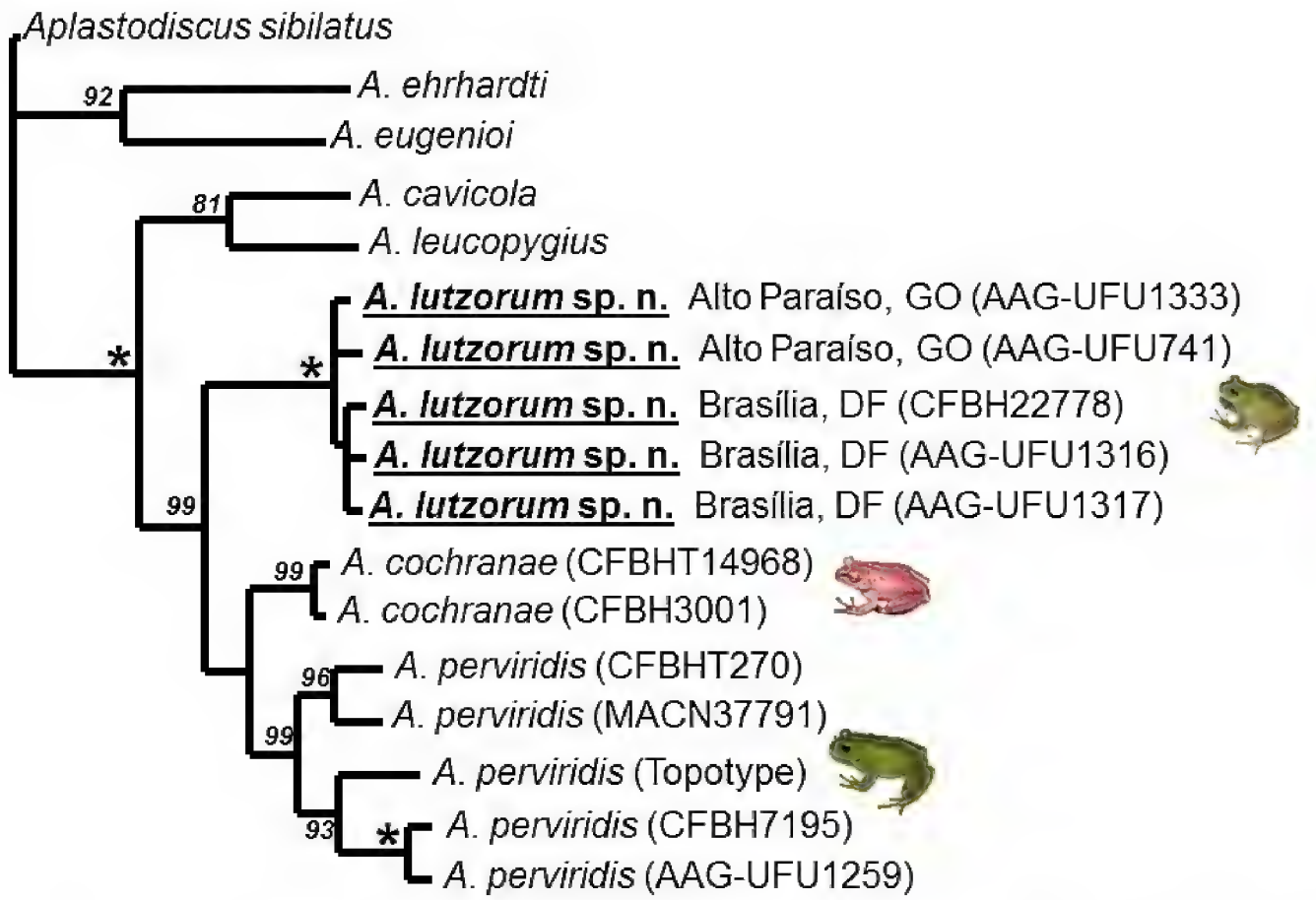

Figure 7. One of the four most parsimonious trees recovered. Asterisks indicate 100% Parsimony Jackknife absolute frequency; only values above 75% are shown. See Suppl. material 2 for details and complete locality names.

ties. *Aplastodiscus lutzorum* sp. n. were recovered as a monophyletic group with 100% Parsimony Jackknife Support (hereafter PJS) being the sister species of *A. perviridis* plus *A. cochranae*. The *A. perviridis* plus *A. cochranae* clade is low supported (54% PJS) and both species are supported by 99% of PJS each. The p-distances calculated for 16s of species in the *A. perviridis* Group range from zero to 5.9% (for all distances see Table 3). The internal distances among specimens of the new species range from zero to 0.93%. The p-distance in 16s between the new species and *A. perviridis* is 4.4–5.8% and between the new species and *A. cochranae* is 4.0–4.5%.

Table 3. Uncorrected pairwise sequence distances (p-distances) of the Cytochrome c oxidase subunit 1 (above the diagonal) and 16s fragments (below the diagonal) for the species of the *Aplastodiscus perviridis* species Group. See the Material and methods section for the primers that delimitate each fragment. Values are in percentage.

		1	2	3	4	5	6	7	8	9	10	11	12
1	*A. cochranae* CFBH3001 Rancho Queimado, SC	—	1.06	9.13	–	–	9.55	8.92	9.77	9.98	9.77	8.92	10.19
2	*A. cochranae* CFBHT14968 Lauro Muller, SC	–	—	9.34	–	–	9.77	9.34	9.98	10.19	9.98	9.13	10.4
3	*A. lutzorum* sp. n. CFBH22778 Brasília, DF	4.28	–	—	–	–	0.42	0.42	10.4	9.34	9.98	9.13	9.55
4	*A. lutzorum* sp. n. AAG1316 Brasília, DF	4.28	–	0.56	—	–	–	–	–	–	–	–	–
5	*A. lutzorum* sp. n. AAG1317 Brasília, DF	4.09	–	0.37	0.19	—	–	–	–	–	–	–	–
6	*A. lutzorum* sp. n. AAG1333 Alto Paraíso, GO	4.09	–	0.37	0.19	0	—	0.85	10.19	9.13	9.77	8.92	9.55
7	*A. lutzorum* sp. n. AAG741 Alto Paraíso, GO	4.46	–	0.93	0.19	0.56	0.56	—	10.4	9.34	9.98	9.13	9.55
8	*A. perviridis* CFBH18119 Topotype	3.35	–	5.2	5.2	5.02	5.02	5.58	—	5.3	6.37	4.03	5.1
9	*A. perviridis* CFBH7195 Santo Antônio do Pinhal, SP	3.16	–	5.39	5.39	5.2	5.2	5.76	2.23	—	6.16	4.25	0.64
10	*A. perviridis* CFBHT270 São Bento do Sul, SC	2.23	–	4.83	4.83	4.65	4.65	5.02	2.79	2.42	—	2.76	5.94
11	*A. perviridis* MACN37791 Misiones, Argentina	3.16	–	5.76	5.76	5.58	5.58	5.95	3.35	3.16	0.93	—	4.03
12	*A. perviridis* AAG1259 Atibaia, SP	2.97	–	5.39	5.39	5.2	5.2	5.76	2.23	0.37	2.42	2.97	—

Discussion

The *A. perviridis* Group now includes a third species, *Aplastodiscus lutzorum*, a species diagnosed mainly by its advertisement call, small size, and genetic differentiation. Genetic p-distances and phylogenetic topology support our hypothesis of the new species. Garcia et al. (2001), when re-describing *A. perviridis*, included six specimens that here we recognize as *Aplastodiscus lutzorum* (CHUNB 404; 268–70; 1378; 1704) the minimum snout-vent lengths values of *A. pervirids* in that work overlaps the SVL of the new species. Garcia et al. (2001) also discuss an unusual condition for any anuran species, observed in *A. perviridis* and *A. cochranae*: identical advertisement calls with clearly distinct coloration (*A. cochranae* is the only brown species of the genus). The description of the advertisement calls of *A. perviridis* in Garcia et al. (2001) were based on specimens from Ribeirão Branco (São Paulo State) and so, do not belong to *A. lutzorum*. The identical advertisement call shared by *A. perviridis* and *A. cochranae* highlight the taxonomic importance of the differences we found in *A. lutzorum*.

Berneck et al. (2016) included only one specimen of the *A. lutzorum* (as *Aplastodiscus* sp. 3), therefore the monophyly of the new species was tested for the first time by

our analysis. Berneck et al. (2016) recovered *Aplastodiscus lutzorum* as a sister species of *A. cochranae*, a topology not corroborated by the present work, where the new species is a sister species of *A. cochranae* plus *A. perviridis*. In the present work, the node of *A. perviridis* plus *A. cochranae* is supported by less than 70% of PJS while in Berneck et al. (2016) the node of *A. lutzorum* plus *A. cochrane* was poorly supported (also less than 70%). Those are possibly the reason of the instability in the internal relationships of members of the *A. perviridis* Group. However, our dataset and taxon sampling is very reduced in relation to that of Berneck et al. (2016) and so the analysis of these authors is preferable for relationships of *Aplastodiscus* species.

The scope of this paper was not to test biogeographic hypotheses. However, *Aplastodiscus lutzorum* is the only species of *Aplastodiscus* that occurs deep in the Cerrado Biome (see Silva et al. 2006) with a disjunctive distribution from its sister species of the Atlantic Forest (Valdujo et al. 2012). Therefore, it seems interesting to point out some remarks on its geographic distribution pattern (Figure 5). The new species has been reported in several localities in the Brazilian Central Plateau and our topology suggests an origin of the *A. perviridis* Group in the Brazilian Central Plateau (Figure 7). However, the low PJF support of the clade *A. perviridis* + *A. cochranae* and the incongruence between our topology and that of Berneck et al. (2016) make any further inference premature.

A population from the dam of Queimado in the municipality of Unaí, in the state of Minas Gerais, Brazil (an area flooded by the construction of a hydroelectric station) was included as "referred specimens" for *A. lutzorum* The conservation status of this population is unknown. We consider the new species to be listed as a "Least Concern", due to the fact that most of its area of occurrence is in protected places, such as the Parque Nacional da Chapada dos Veadeiros, Área de Relevante Interesse Ecológico (ARIE) do Capetinga/Taquara (Fazenda Água Limpa), Estação Ecológica de Águas Emendadas, and Floresta Nacional de Silvânia.

Goin (1961) suggested that *Chorophilus cuzcanus* Cope, 1878 should be an *Aplastodiscus*, but had not stated that it was *Aplastodiscus perviridis* (as pointed out by Frost, 2016). Lutz (1968) suggested that *C. cuzcanus* was possibly a second species of *Aplastodiscus* at that time. According to Frost (2016), the combination *Chorophilus cuzcanus* is a junior synonym to both *A. perviridis* and *Gastrotheca marsupiata* (Duméril and Bibron 1841). We recognize only the synonym of Duellman and Fritts (1972) for *G. marsupiata* as valid; therefore *A. perviridis* has no junior synonyms.

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References

Bastos RP, Motta JAO, Lima LP, Guimarães LDA (2003) Anfíbios da Floresta Nacional de Silvânia, Estado de Goiás. Goiânia, Brazil, 29 pp.

Berneck BVM, Haddad CFB, Lyra ML, Cruz CAG, Faivovich J (2016) The Green Clade Grows: a phylogenetic analysis of *Aplastodiscus* (Anura; Hylidae). Molecular Phylogenetics and Evolution 97: 213–223. https://doi.org/10.1016/j.ympev.2015.11.014

Bioacoustics Research Program (2014) Raven Pro: Interactive Sound Analysis Software (Version 1.5) [Computer software]. The Cornell Lab of Ornithology, Ithaca, NY. http://www.birds.cornell.edu/raven

Brandão RA, Araujo AFB (2002) A herpetofauna da Estação Ecológica de Águas Emendadas. In: Marinho-Filho JS, Rodrigues FC, Guimarães MM (Eds) Vertebrados da Estação Ecológica de Águas Emendadas, História Natural e Ecologia em um Fragmento de Cerrado do Brasil Central. Brasília, 92 pp.

Charif RA, Strickman LM, Waack AM (2010) Raven Pro 1.4 User's Manual. The Cornell Lab of Ornithology, Ithaca, NY. http://www.birds.cornell.edu/raven

Duellman WE (1970) The hylid frogs of Middle America. Monograph of the Museum of Natural History, University of Kansas 1–2: 1–753.

Duellman WE, Fritts TH (1972) A taxonomic review of the southern Andean marsupial frogs (Hylidae: *Gastrotheca*). Occasional Papers of the Museum of Natural History 9: 1–37.

Farris JS, Albert VA, Källersjö M, Lipscomb D, Kluge AG (1996) Parsimony jackknifing outperforms neighbor-joining. Cladistics 12: 99–124. https://doi.org/10.1111/j.1096-0031.1996.tb00196.x

Frost DR (2016) Amphibian Species of the World: An Online Reference. Version 6.0 (September, 4, 2016). American Museum of Natural History, New York. Electronic Database accessible at http://research.amnh.org/herpetology/amphibia/index.html

Garcia PCA, Caramaschi U, Kwet A (2001) O status taxonômico de *Hyla cochranae* e recaracterização de *Aplastodiscus* A. Lutz (Anura, Hylidae) Revista Brasileira de Zoologia 18: 1197–1218. http://dx.doi.org/10.1590/S0101-81752001000400015

Goin CJ (1961) Synopsis of the genera of hylid frogs. Annals of the Carnegie Museum 36: 5–18.

Goloboff PA (1999) Analyzing large datasets in reasonable times: solutions for composite optima. Cladistics 15: 415–428. https://doi.org/10.1111/j.1096-0031.1999.tb00278.x

Goloboff PA, Farris JS, Nixon KC (2008) TNT, a free program for phylogenetic analysis. Cladistics 24: 1–13. https://doi.org/10.1111/j.1096-0031.2008.00217.x

Heyer WR, Rand AS, Cruz CAG, Peixoto OL, Nelson CE (1990) Frogs of Boracéia. Arquivos de Zoologia 31: 231–410. http://dx.doi.org/10.2307/1446606

Jungfer KH, Faivovich J, Padial JM, Castroviejo-Fisher S, Lyra ML, Berneck BVM, Iglesias PP, Kok PJR, MacCulloch RD, Rodrigues MT, Verdade VK, Torres-Gastello CP, Chaparro JC, Valdujo PH, Reichle SM, Gvoždík V, Gagliardi-Urrutia G, Ernst R, De la Riva I, Means DB, Lima AP, Señaris JC, Wheeler WC, Haddad CFB (2013) Systematics of spiny-backed treefrogs (Hylidae: *Osteocephalus*): an Amazonian puzzle. Zoologica Scripta 42: 351–380. https://doi.org/10.1111/zsc.12015

Lutz B (1950) Anfíbios anuros da coleção Adolpho Lutz do Instituto Oswaldo Cruz. V. Frogs in the Adolpho Lutz collection of the Instituto Oswaldo Cruz. V. Memórias do Instituto Oswaldo Cruz 48: 599–637. https://doi.org/10.1590/S0074-02761950000100022

Lutz B (1968) Taxonomy of the Neotropical Hylidae Pearce-Sellards Series Texas Memorial Museum 11: 3–26.

Morais AR, Bastos RP, Vieira R, Signorelli L (2012) Herpetofauna da Floresta Nacional de Silvânia, um remanescente de Cerrado no Brasil Central. Neotropical Biology and Conservation 7: 114–121. https://doi.org/10.4013/nbc.2012.72.05

Myers CW, Duellman WE (1982) A new species of *Hyla* from Cerro Colorado, and other tree-frog records and geographical notes from Western Panama. American Museum Novitates 2752: 1–32.

Palumbi SR, Martin A, McMillan WO, Stice L, Grabowski G (1991) The simple fool's guide to PCR, Version 2.0. http://palumbi.stanford.edu/SimpleFoolsMaster.pdf

Rambaut A (2014) FigTree, ver. 1.4.2. (Online 2016, May 29). http://tree.bio.ed.ac.uk/software/figtree/

Santoro GRCC, Brandão RA (2014) Reproductive modes, habitat use, and richness of anurans from Chapada dos Veadeiros, central Brazil. North-Western Journal of Zoology 10: 365–373. http://biozoojournals.ro/nwjz/content/v10n2.html

Savage JM, Heyer, WR (1967) Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. Beiträge zur Neotropischen Fauna 5: 111–131. http://dx.doi.org/10.1080/01650526709360400

Silva JF, Fariñas MR, Felfili JM, Klink CA. (2006) Spatial heterogeneity, land use and conservation in the Cerrado region of Brazil. Journal of Biogeography 33: 536–548. doi:10.1111/j.1365-2699.2005.01422.x

Sueur J, Aubin T, Simonis C (2008) Seewave: a free modular tool for sound analysis and synthesis. Bioacoustics 18: 213–226. https://doi.org/10.1080/09524622.2008.9753600

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725–2729. https://doi.org/10.1093/molbev/mst197

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting. position-specific gap penalties and weight matrix choice Nucleic Acids Research 22: 4673–80. https://doi.org/10.1093/nar/22.22.4673

Valdujo PH, Silvano DL, Colli G, Martins M (2012) Anuran species composition and distribution patterns in Brazilian Cerrado, a Neotropical hotspot. South American Journal of Herpetology 7: 63–78. http://dx.doi.org/10.2994/057.007.0209

Wilkinson JA, Matsui M, Terachi T (1996) Geographic variation in a Japanese tree frog (*Rhacophorus arboreus*) revealed by PCR-aided restriction site analysis of mtDNA. Journal of Herpetology 30: 418–423. https://doi.org/10.2307/1565184

Supplementary material 1

Analyzed sound files; from Ariovaldo A. Giaretta collection

Authors: Bianca V. M. Berneck, Ariovaldo A. Giaretta, Reuber A. Brandão, Carlos A. G. Cruz, Célio F. B. Haddad

Data type: species data

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Supplementary material 2

List of voucher specimens, GenBank accession numbers, and locality data

Authors: Bianca V. M. Berneck, Ariovaldo A. Giaretta, Reuber A. Brandão, Carlos A. G. Cruz, Célio F. B. Haddad

Data type: species data

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